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FUNGI AND HYDROCARBONS IN THE
MARINE ENVIRONMENT

by

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FUNGI AND HYDROCARBONS IN THE MARINE ENVIRONMENT
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Hydrocarbons from various sources--anthropogenic pollution, marine seeps, marine algae, atmospheric fallout and terrestrial runoff--enter the ocean daily. These complex hydrocarbon mixtures are dispersed and degraded by abiotic and biogenic processes (Floodgate 1984). The rate of degradation and the significance of microbial activities in the fate of oceanic hydrocarbons vary with environmental conditions and the type of hydrocarbon. Most commonly, bacteria are considered the primary degraders, with algae and fungi having minor roles. Although implied in a number of cases, the degradation of complex hydrocarbon mixtures by a successional microflora containing temporally isolated populations of bacteria and fungi, has been inadequately studied.

METABOLISM

The majority of detailed metabolic studies on hydrocarbon metabolism by fungi have been carried out with geophilic yeasts (Shenan & Levi 1974); however, a variety of fungi from marine habitats also possess the capacity to metabolize hydrocarbons (Ahearn et al. 1971; Cerniglia 1984). These diverse groups of fungi commonly utilize short-chained n-alkanes (C8-C18). As in most microorganisms, the primary route of metabolism in the fungi is monoterminal oxidation to the corresponding alcohol, aldehyde and fatty acid. The fatty acid may then be oxidized to acetate through beta oxidation or may be incorporated into cellular lipids (Singer & Finnerty 1984). Finnerty and co-workers (Modrzakowski et al. 1977; Modrzakowski & Finnerty 1980) have suggested an alternate pathway for alkane oxidation from evidence employing the alkane analog di-octyl ether. We have been unable to demonstrate growth on dioctyl ether by yeasts, although several species of *Candida* are capable of growth on the C6 and C10 analogs. Diterminal oxidation yielding diterminal fatty acids is known (Rehm & Reiff 1981). Here the monocarboxylic fatty acid is oxidized to the ω -hydroxy fatty acid and then to the dicarboxylic fatty acid. Certain fungi appear to oxidize alkanes exclusively by subterminal oxidation to secondary alcohols and then to a ketone (Rehm and Rieff 1981). Alkenes are oxidized at sites of unsaturation as well as terminally. Branched chain hydrocarbons such as pristane (2,6,10,14-tetramethylpentadecane) are oxidized or co-oxidized by certain yeasts (Hagihara et al. 1977). We have found that all of the 37 isolates of *Candida lipolytica* in our collection, including 12 isolates of marine origin, assimilated pristane as a sole

source of carbon for growth. *Candida lipolytica* also grew with squalene (although more slowly than with pristane) as the substrate (unpublished data). *Candida maltosa* did not utilize these substrates as a sole source of carbon for growth but apparently co-oxidized pristane in the presence of tetradecane (Crow et al. 1980).

Few fungi metabolize cycloalkanes. Komagata et al. (1974) examined 498 yeast strains and found that none was capable of utilizing cyclopentane. In our unpublished studies, cycloalkanes have not appeared toxic to representative yeasts. The metabolism of cycloalkanes in the fungi apparently proceeds via cooxidation. Oxidation of aromatic compounds such as phenol, salicylate, benzoate and mandelate has been found to be an inducible property of *C. tropicalis*, *Trichosporon* spp. and *Rhodotorula* spp., yeasts that represent hydrocarbonoclastic taxa. Various species of these genera oxidize mandelate and benzoate via the protocatechuate branch and salicylate by the catechol branch of the β -ketoadipate pathway (Durham et al. 1984, Mortberg & Neujahr 1985). We have found that isolates of *Rhodotorula* and *Trichosporon* grow in sediment and water samples from coastal habitats that have been enriched with low levels of glucose ($<2 \mu\text{M}$) and phenol ($5-15 \mu\text{M}$) but species of *Candida* with at least some fermentative capacity become predominant more often at glucose levels of $5 \mu\text{M}$ or greater or with the addition of 0.1% yeast extract (unpublished data). Polycyclic aromatic hydrocarbons (PAHs) are oxidized by a variety of fungi and molds from the marine environment (Cerniglia & Crow 1981; Cerniglia 1984). PAHs are apparently oxidized via a cytochrome P-450 monooxygenase to arene oxides that either isomerize to phenols or are enzymatically hydrated to transdihydriols.

DISTRIBUTION OF MARINE FUNGI

Fungi that are endemic to the marine environment are found mostly in the euphotic zone, particularly in littoral regions. A few cellulolytic species are found to depths of about 1000m; but in general, marine fungi seem to be rare in the ocean depths (Kohlmeyer & Kohlmeyer 1979). Kohlmeyer (1983) stated that the major factors controlling the distribution of marine fungi were availability of substrates or hosts, temperature, hydrostatic pressure, and oxygen. However *in situ* evidence that fungi express hydrocarbonoclastic activity in the natural environment is sparse. Mostly, evidence of activity is indirect; implied on the basis that a variety of filamentous fungi, many of which are known to degrade hydrocarbons, has been isolated from the oceans. Marine arenicolous and lignicolous fungi have not been investigated for hydrocarbon degradation, and there is no evidence that associates any of these fungi with hydrocarbon enrichment.

The ubiquitous presence of yeasts in the oceans has been amply documented (Hagler & Ahearn 1985). Apparently most yeasts enter the oceans with terrestrial runoff. *Debaryomyces hansenii*, the *Candida parapsilosis* complex, and *Rhodotorula* spp., for example, seem physiologically adapted for survival in low nutrient conditions and increase in density upon their fortuitous encounter with regions of

increased nutrients (Ahearn & Meyers 1976; Fell 1976). *Metschnikowia* spp. may be commensals and pathogens for planktonic crustaceans (Seki & Fulton 1969). Yeast densities may be particularly high (10^3 - 10^5 ml⁻¹) in surface films (Crow et al. 1975; Crow et al. 1976) and in estuarine marshes ($>9 \times 10^4$ cm⁻³) (Ahearn et al. 1976). Increased densities of yeasts in offshore water have been associated with algal blooms (Meyers et al. 1967) and it is probable that yeasts are active in the decomposition of algal products (Gunkel et al. 1983).

ASSOCIATION OF FUNGI WITH OIL

A few geophilic molds, with hydrocarbonoclastic capacities such as *Aspergillus versicolor* and *Cladosporium* spp. show widespread oceanic distribution (Roth et al. 1963; Muntanola-Cvetkovic & Risljanovic 1980). Geophilic hyphomycetes that are known to develop on decaying organic matter in littoral zones (Kirk 1983) include hydrocarbonoclastic species (Bossert & Bartha 1984). The role of these filamentous fungi in the biodegradation of hydrocarbons in the oceans is unclear. In reviewing factors that may account for the paucity of information on the environmental role of molds in the degradation of hydrocarbons in freshwater, Cooney (1984) indicated that most environmental studies have centered on bacteria with media and other conditions unfavorable for optimal isolation of molds or for their utilization of hydrocarbons. These observations may be applied to some marine studies. Fedorak et al. (1984) reported that only 29% of 224 molds from a marine environment altered the n-alkane profile of crude oil. Gruttner & Jensen (1983) found that mold populations in a marine coastal habitat tended to be higher with proximity to an oil refinery. Over 90% of the isolates were able to grow with oil as the only carbon source. Fungi in hydrocarbon enriched littoral areas usually seem to develop secondary to bacteria. In samples from certain of our hydrocarbon enriched ecocores (Spain & Somerville 1985), molds developed only after several months (unpublished data). This delay in fungal development has been noted also for samples collected from the Amoco Cadiz spill (Ahearn & Crow 1980).

Yeasts and fungi of the genera *Acremonium*, *Aureobasidium*, and *Cladosporium*, which may produce single budding cells under certain conditions, have been associated occasionally (but more often than molds) with oil pollution of marine habitats. LePetit et al. (1970) found that two hydrocarbonoclastic species, *Candida tropicalis* and *C. lipolytica*, occurred in increased densities in littoral areas influenced by effluents from an oil refinery. *Candida lipolytica*, one of the most active hydrocarbonoclastic yeasts, was isolated from the vicinity of wells in the North Sea (Crow et al. 1977). The rare occurrence of this species in sea water suggests the presence of hydrocarbons (LePetit et al. 1970).

In marshlands dominated by *Spartina alterniflora*, the native yeast species *Pichia spartina* and *Kluyveromyces drosophilae*, which are not hydrocarbonoclastic, were replaced gradually after the addition of oil by species of *Rhodotorula*, *Trichosporon*, and *Pichia* that utilized

alkanes for growth (Ahearn et al. 1976). Candida tropicalis was found to be associated with inshore waters of Biscayne Bay, Florida, but it was not observed to occur in increased densities in proximity to occasional oil slicks (Ahearn 1973). In offshore waters, Rhodotorula glutinis and C. parapsilosis showed increased densities in surface waters influenced by a spewing oil well; however, these increased densities were not observed in a repeated sampling several weeks later even though oil was still present (Ahearn et al. 1971). A possible influence of offshore drilling activities on yeast flora in the North Sea was noted in the comparison of species isolated in 1964-66 with species isolated in 1976 (Ahearn & Crow 1980). Debaryomyces hansenii, which shows slow growth on short-chained alkanes, was the predominant species in both sets of samples; but after oil production, Candida guilliermondii, a hydrocarbonoclastic yeast, was encountered three times more frequently. Successional development of yeast species with more hydrocarbonoclastic species--Candida tropicalis and Candida guilliermondii replacing the early developing Rhodotorula--has been observed in samples of beached oil from the Amoco Cadiz (Ahearn & Crow 1980).

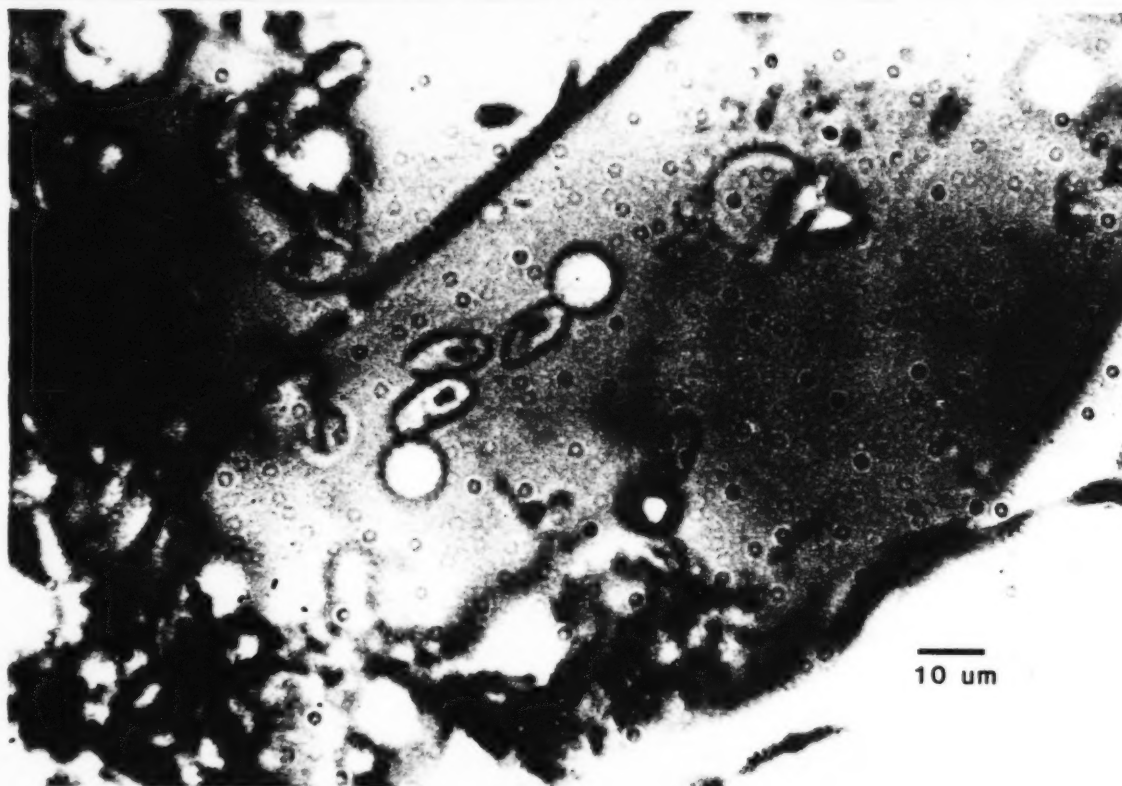
Yeasts were not observed with an enriched culture medium in our laboratory studies of ground water tainted with gasoline leaking from a storage tank; however, with basal salts agar and extended incubation, Candida guilliermondii at densities of $10^3 - 10^4$ cells L^{-1} was recovered (Cook & Raymond unpublished). The more volatile hydrocarbons may alter the plasma membrane and, dependent upon concentration, may either stimulate or inhibit growth (Ahearn et al. 1971; Gill & Ratledge 1972). The emergence of hydrocarbonoclastic fungi also may be dependent on shifts in pH. In enclosed bunkers of oil tankers that employed seawater compensation systems, approximately 20% of 80 systems were dominated by Cladosporium resinae and Candida spp. (Neihof & May 1983). Cladosporium resinae grows slowly at the pH of seawater, and organic acid accumulation may be necessary for stimulation of growth (Neihof & May 1983). Microhabitats dominated by fungi may occur in water in oil emulsions and may result in transient periods of yeast and mold degradation of crude oil in marine habitats (Fig. 1).

UPTAKE OF HYDROCARBONS

Singer & Finnerty (1984) state that uptake of alkane by yeasts may involve: (1) adsorption to the cell via hydrophobic surface projections, (2) movement through the rigid cell wall via pores or channels with initial accumulation on the outer surface of the cell membrane, and/or (3) movement of the alkane via pinocytosis to microbodies and other sites of oxidation (intra-cytoplasmic inclusions may also be formed). The immediate steps in hydrocarbon uptake are probably energy independent, but facilitated by various complexes of fatty acids with protein and (or) carbohydrates. Kappeli & coworkers have extensively examined the uptake and transport of hydrocarbons in C. tropicalis (Kappeli & Fiechter 1977; Kappeli et al. 1978; Kappeli & Fiechter 1981). The organism produced a mannan-fatty acid complex that rapidly emulsified the hydrocarbon and induced significant uptake of

pure hydrocarbon. *Candida lipolytica* has also been shown to produce an extracellular product capable of inducing both emulsification and uptake of hydrocarbons (Singer & Finnerty 1984). These bioemulsifiers vary in nature or quantity with the particular yeast.

Figure 1. Yeast and mold growth in smear of beached oil collected from the coast of Louisiana.



COMPENDIUM

While the natural populations of yeasts and molds in open ocean water would seem to be insufficient to produce significant alterations of the composition of crude oils, unique areas such as the surface film, decomposing algae, or tar-balls may contain highly active levels of fungi. The action of the fungi in these habitats may be noteworthy. During the process of growth on crude oil or mixed hydrocarbons, simultaneous transport of hydrocarbons may occur, resulting in enhanced oxidation of essentially nonwater soluble aromatics such as biphenyl and naphthalene or branch chained alkanes such as pristane. Crow & Bell (1981) have observed the uptake of naphthalene by cells grown on tetradecane and naphthalene. Furthermore, significant uptake of pristane was observed in a yeast incapable of growth on this substrate. The uptake and release of compartmentalized hydrocarbons from eukaryotic microorganisms remains an unquantitated factor in the fate of hydrocarbons in the marine environment.

The observed associations do not provide definitive evidence that fungi degrade oils in the marine environment. Nevertheless, the occasional association of certain species with oil enrichment, particularly successional habitation, coupled with knowledge that these developing taxa possess varied hydrocarbonoclastic activities, suggests an active role for fungi. Often fungi do not attain significant densities in an oil-enriched habitat until at least several months after the addition of oil. Since the abiotic parameters of volatilization and aquatic dispersion provide, in the main, for the relatively fast disappearance of oil in open waters, the sites for hydrocarbon degradation by fungi in marine habitats would probably be best found where oil is transported to coastal habitats. Further studies are necessary to elucidate the role of fungi in hydrocarbon degradation in the marine environment.

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